

D0128 NP

Figure 1A

1 CCACGCGTCCGCTCAGGTCTATAGGATTAAGAAAGGCAAGCCCAGCAGCCACTACTCACT 60
 61 GACCAGACCTGGCCCAACATGCTGCAGAAATAATTATCAATTAGTATACTTGAGAGACAG 120
 121 CAGCGTGAGGTGGAGAATGGGTTCTAAACTGAATGACAGCTGTTAACAGTTTTTTGGCCCT 180
 181 GTTTTTCTCTGCTGAATCCTCAACTGAGATCCTAGGGATGAGAAACGGGGGAACAGCAC 240
 241 GCCCTACTTGAGAGAATTAGAATTTGAGGCGCTAGGAAGCAAAGGATCCCAAAGATGGC 300
 301 GACCTGCCAGCCTGGACTGCCAGCGAAGGCCAGAATCGTGCTGTAGCTCTGAACCCACAG 360
 361 CTCCTCTGCCCTGGCCCATGAGAATTTAGCTGGAGAGATAGCATGCCCTGGTAAGTGA 420
 421 AGTCCTGCCACTTCGAGACATGGAATCATCTTTCTCATTTGGAGTGATCCTTGCTGCTCCT 480
 1 M E S S F S F G V I L A V L 14
 481 GGCCTCCCTCATCATTGCTACTAACACACTAGTGGCTGTGGCTGTGCTGCTGTTGATCCA 540
 15 A S L I I A T N T L V A V A V L L L I H 34
 541 CAAGAATGATGGTGTGTCAGTCTCTGCTTCACCTTGAATCTGGCTGTGGCTGACACCTTGAT 600
 35 K N D G V S L C F T L N L A V A D T L I 54
 601 TGGTGTGGCCATCTCTGGCCTACTCACAGACCAGCTCTCCAGCCCTTCTCGGCCCCACACA 660
 55 G V A I S G L L T D Q L S S P S R P T Q 74
 661 GAAGACCCTGTGCAGCCTGCGGATGGCATTGTGCACTTCCTCCGAGCTGCCTCTGTCCCT 720
 75 K T L Q S L R M A F V T S S A A A S V L 94
 721 CACGGTCATGCTGATCACCTTTGACAGGTACCTTGCCATCAAGCAGCCCTTCCGCTACTT 780
 95 T V M L I T F D R Y L A I K Q P F R Y L 114
 781 GAAGATCATGAGTGGGTTTCGTGGCCGGGGCTGCATTGCCGGGCTGTGGTTAGTGTCTTA 840
 115 K I M S G F V A G A C I A G L W L V S Y 134
 841 CCTCATTTGGCTTCCTCCCACTCGGAATCCCCATGTTCCAGCAGACTGCCTACAAAGGGCA 900
 135 L I G F L P L G I P M F Q Q T A Y K G Q 154
 901 GTGCAGCTTCTTTGCTGTATTTACCCCTCACTTCGTGCTGACCCTCTCCTGCGTTGGCTT 960
 155 C S F F A V F H P H F V L T L S C V G F 174

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Figure 1B

961	CTTCCCAGCCATGCTCCTCTTTGTCTTCTTCTACTGCGACATGCTCAAGATTGCCTCCAT	1020
175	<u>F P A M L L F V F F Y</u> C D M L K I A S M	194
1021	GCACAGCCAGCAGATTTCGAAAGATGGAACATGCAGGAGCCATGGCTGGAGGTTATCGATC	1080
195	H S Q Q I R K M E H A G A M A G G Y R S	214
1081	CCCACGGACTCCCAGCGACTTCAAAGCTCTCCGTACTGTGTCTGTTCTCATTGGGAGCTT	1140
215	P R T P S D F K A L R <u>T V S V L I G S F</u>	234
1141	TGCTCTATCCTGGACCCCCTTCCTTATCACTGGCATTGTGCAGGTGGCCTGCCAGGAGTG	1200
235	<u>A L S W T P F L I T G I V</u> Q V A C Q E C	254
1201	TCACCTCTACCTAGTGCTGGAACGGTACCTGTGGCTGCTCGGCGTGGGCAACTCCCTGCT	1260
255	H L Y L V L E R <u>Y L W L L G V G N S L L</u>	274
1261	CAACCCACTCATCTATGCCTATTGGCAGAAGGAGGTGCGACTGCAGCTCTACCACATGGC	1320
275	<u>N P L I Y A Y W</u> Q K E V R L Q L Y H M A	294
1321	CCTAGGAGTGAAGAAGGTGCTCACCTCATTCTCTCTTTCTCTCGGCCAGGAATTGTGG	1380
295	L G V K K V L T S F L L F L S A R N C G	314
1381	CCCAGAGAGGCCAGGGAAAGTTCTGTCAATCGTCACTATCTCCAGCTCAGAGTTTGA	1440
315	P E R P R E S S C H I V T I S S S E F D	334
1441	TGGCTAAGACGTCGCTTTGCTTACCAGTCTGGCCCAGAGGAGAAACATGCTTGCTTTTAC	1500
335	G	335
1501	CATAGCAAGCATCGTGTTCTTACAACCTGAAGAACTTTTTGACTCTCAAGTGGACGATGGA	1560
1561	ACCCAATATGCCTTGAAATGCTGAGCATCCAGCAAGTACCAGGCCAGCAATAAGGCACAT	1620
1621	TCTTGGATTGTATTGTGAGTTACTGGGAACTAGGTAAAAATACGAATAACTGGATTTTGG	1680
1681	CAAAATTGAAAGCAGGCTCAAACCAATGCCCTCCTTACTGGTGGTTCAAAGCTTAAATCC	1740
1741	TGGCCTTGCTACAAAGGACTGATCTTGCCAAGATAATATAAGCAAAGTGGAATGAAAATT	1800
1801	AAAGTTAATTCTGAAGCCAAGGTCTTTTAGAAAAAAAAAAGTAAATTAGCCTGATTTAA	1860
1861	TCATTCCACATTTTAAACATATATCAACATCACATTGTACCCACGTAATATATACAATT	1920
1921	ATTTGTCAATTAAAAATAAAAAATATATATTTTTTAAGTACAGAAAAGCAGGAGGGGAGGA	1980

1981	GAGGCCAGGTGATCAGTGTGTTATTAGGTTCAATCTAAGAAACTCTGCTACCCCTGGGAG	2040
2041	TCAGTATGTGCAATGGAAAAATCACAGGTCTTGAAATCCAACAGACCAGTGTTCAAATGA	2100
2101	TAATTCAGTCATTTAAATTCTCAAACCTAGATCTCGCATTCATCAAACCGGGGTAATAATG	2160
2161	CCTACTTTACATGGTTTATATTGAAGATTAACCTCAGATAATGTATATGTAAATATCTAGTA	2220
2221	AACTACAGCACATTGTTAGTGCTCAAAAAAAAAAAAAAAAAAAG	2260

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Figure 2A

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B2AR_CANFA ~~~~~MGQPANRSVFLAPNGSHAPDQGDSQERSEAWV.VGMCIVMSLIVLAIVFGNVL
B2AR_PIG ~~~~~MGQPGNRSVFLAPNGSHAPDQDVQERDEAWV.VGMAIVMSLIVLAIVFGNVL
B2AR_MOUSE ~~~~~MGPHGNDSDFLAPNGSRAPDHDVTQERDEAWV.VGMAILMSVIVLAIVFGNVL
B4AR_MELGA ~~~~~~MTPLPAGNGS.VPNCSWAAVLSRQWA.VGAA..LSITILVIVAGNLL
D1A2_EEL ~~~~~~MDLNFSTVLDS.GLSET.DSSVRVLTGCFLLSSLIIVSTLLGNTL
D1BR_CARP ~~~~~~MRAPRS.GAQHA.RPN.RA.AGELTRALILWTLGNTL
D5DR_FUGRU MENFYNETEPTPEPRGGVDPLRVVTAEDVPAPVG.GVSVRALTGCVLCALIVSTLLGNTL
D1B_AMPHIOXUS ~MSANTTVSPTETTANLTANSTEASVGSCFAPNPYSAGVQAVLGLITVILILLTVIGNVL
5H4_CAVPO ~~~~~~MDKLDANVSSKEGFGSVEKVLLTFLSAVILMAILGNLL
HGPRBMY26 ~~~~~~MESSFSFGVILAVLASLITATNTL

B2AR_CANFA VITAIARFERLOT.VTNYFITSLACADLVMLAVVPPGASHILMKMWTFGNFWCEFWISI
B2AR_PIG VITAIKFERLOT.VTNYFITSLACADLVMLAVVPPGASHILMKMWTFGNFWCEFWISI
B2AR_MOUSE VITAIKFERLOT.VTNYFITSLACADLVMLAVVPPGASHILMKMWNFGNFWCEFWISI
B4AR_MELGA VIVAIKTPRLQT.MTNVFTVSLACADLVMLLVPPGATILLSCHWPYGTGVCELTSL
D1A2_EEL VCAAVTKFRHLRSKVTNFFVIVSLAVSDLLVAILVMPWKAVTEVAGFWPFGSF.CNIWVAF
D1BR_CARP VCATVVRFRHLRAKVTHVFISLAVSDLLVAVLVMPWKAVAEVAGFWPFGAF.CNIWVAF
D5DR_FUGRU VCAAVIKFRHLRSKVTNAFVIVSLAVSDLVAVLVMPWRRAVSEVAGVWLFGRF.CDTWVAF
D1B_AMPHIOXUS VILAVTCHRFKMT.VTNFFIVSLACADLSVGLTVLPFAATNDLLGYWPFPGGY.CDVWVSE
5H4_CAVPO VMVAVCRDRQLRKIKTNYFIVSLAFADLLVSVLVMPFGAIELVQDIWVYGEMFCLVRTSL
HGPRBMY26 VAVAVLLLHKNDGVSLCFTLNLAVADTLIGVATSGLLTDQLSSPSRPTQKTLCSLRMAF

B2AR_CANFA DVLCVTASITLTCVIAVDRYFAI.TSPFKYQSLLTKNKARVVILMVWIVSGLTSFLPIQM
B2AR_PIG DVLCVTASITLTCVIAVDRYLAI.TSPFKYQCLLTKNKARVVILMVWVVSGLISFLPIKM
B2AR_MOUSE DVLCVTASITLTCVIAVDRYVAI.TSPFKYQSLLTKNKARVVILMVWIVSGLTSFLPIQM
B4AR_MELGA DVLCVTASITLTCVIAVDRYFAI.TAPLOYEALVTKGRAWAVCMVWVAISAFISFLPIMN
D1A2_EEL DIMCSTASILNLCIISVDRYVAI.SSPFRYERKMTPKVAFVMTSVAVTLLSILISFIPVOL
D1BR_CARP DIMCSTASILNLCVISVDRYVAI.SSPFRYERKMTPRVSFVMTGAATLSVLISFIPVOL
D5DR_FUGRU DIMCSTASILNLCVISMDRYVAI.SNPFYERRMTRRFALMTIAVAVTLLSVLISFIPVOL
D1B_AMPHIOXUS DVLNSTASILNLVIAFDREFAI.TAPFTYHTRMTERTAGILIAVWGISLVVSFLPIQA
5H4_CAVPO DVLLTTASIFHLCCISLDRYVAICCPVLVYRNKMTPLRIALMLGGCWVIFPMFISFLPIMQ
HGPRBMY26 VTSSAAASVLTVMILITFDRYLAI.OPFRYLKIMSGFVAGACTAGLWLVSYLIGFLPLGI

B2AR_CANFA HWYR...ATHQEAICYAKETC..CDFFTNQAYAIASSIVSFYLPVVMVVFVYSRVFQVA
B2AR_PIG HWYQ...ATHREALNCYAEAC..CDFFTNQPYAIASSIVSFYLPVVMVVFVYSRVFQVA
B2AR_MOUSE HWYR...ATHKKAIDCYTEETC..CDFFTNQAYAIASSIVSFYLPVVMVVFVYSRVFQVA
B4AR_MELGA HWWR...DGADEQAVRCYDDPRC..CDFVTNMTYAIVSSTVSFYVPLLVMIFFVYVRVFAVA
D1A2_EEL NWHKAQTTSYFDHNGSYGDLNCDSSLRNTYAISSSLISFYIPVAIMVITYTRYRIA
D1BR_CARP DWHKTDAGA.AEPNAS..D..ADSCDSSLRNVYAISSSLISFYIPVAIMVITYTRYRIA
D5DR_FUGRU NWHRADNNSAHEQG.....DCNASLRNTYAISSSLISFYIPVIMVGYTRYTRIFRIA
D1B_AMPHIOXUS GWYR.DNOSE.EALAIYSDPCL..CIFTASTAYTISSSLISFYIPLLIMLVFYGIIFKAA
5H4_CAVPO GWNIGIVDLTEKRKFQNSNSTYCVFMVNKPYAITCSVVAFYIFFLMLVLAYRYIYVTA
HGPRBMY26 PMEQQT.....AYKGQCSFFAVFHPH..EVLTLSCVGFFPAMLLFVFFFYCDMLKIA

B2AR_CANFA QRLOKIDRSE.GRFHAQNLSQVEQDGRSGHGHRRSSK.FCLKEHKALKTLGIIMGTFTL
B2AR_PIG RRLOKIDKSE.GRFHAQNLSQAEQDGRSGPGHRRSSK.FCLKEHKALKTLGIIMGTFTL
B2AR_MOUSE KRLOKIDKSE.GRFHAQNLSQVEQDGRSGHGLRRSSK.FCLKEHKALKTLGIIMGTFTL
B4AR_MELGA TRHVQLIGKDK.VRFLQENPSLSRGGR...WRFPRLAIKEHKALKTLGIIMGTFTL
D1A2_EEL QKQIRRIASLERAA.ESAKNRHNSMGNSSSVETESSFKMSFKRETKVLKTLVIMGVFVC
D1BR_CARP QVCIRRIASLERAA.EHAQSR...SDRSLHRSIKTSFQRETKVLKTLSVIIGVFVC
D5DR_FUGRU QTQIRRISSLERAAAGQRAQNO...SHRASTHDESALKTSFKRETKVLKTLVIMGVFVF
D1B_AMPHIOXUS RDQARKINALE.GELEQENNRGK.....KISLAKEKKAAKTLGIIMGVFVL
5H4_CAVPO KEHARQIQVLQAGAPAE.....GRPOPADQHSRTHR.MRTETKAAAKTLGIIMGCFCL
HGPRBMY26 SMHSQQIRKMEHAGAMA.....GGYRSPT..PSDFKALRTVSVLIGSFAL

B2AR_CANFA CWLPFFIIVNIVHVIQD.....NLIPKEVYILLNW.VGYVNSAFNPLIYCR.SPDF
B2AR_PIG CWLPFFIIVNIVHGIHD.....NLIPKEVYILLNW.VGYVNSAFNPLIYCR.SPDF
B2AR_MOUSE CWLPFFIIVNIVHVIRD.....NLIPKEVYILLNW.LGYVNSAFNPLIYCR.SPDF
B4AR_MELGA CWLPFFVANIKVFCR.....PLVPDQLFLFLNW.LGYVNSAFNPLIYCR.SPDF
D1A2_EEL CWLPFFILNCMVPFCEQAHPNGSADFPVCSSTTFNVFVW.FGWANSINPLIYAF.NADF
D1BR_CARP CWLPFFVLNCPVPGRRE.....PCVTDTTDFVFW.FGWSNSSLNPVIYAF.NAEF
D5DR_FUGRU CWLPFFVLNCPVPFCD...VDKVGEPVCSDTTFNVFVW.FGWANSINPLIYAF.NADF
D1B_AMPHIOXUS CWLPFFVNVNVPFCDR.....CVQPAVEIALTW.LGWINSCNPLIYAF.NKEF
5H4_CAVPO CWAPFFVTNIVDPFIDYT.....VPGQLWTAFLW.LGYINSGLNPFLIYAF.NKSE
HGPRBMY26 SWTPFLITGIVQVACQECHLYLVLER.....YLWLLGVGNSLLNPLIYAYWQKEV

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. Figure 2B

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B2AR_CANFA  R.IAFQELLCLRRSSLKAYGNGYSNNSNSRSDYAGEHSGCHLGQEKDS..ELLCEDPFGT
B2AR_PIG     R.MAFQELLCLHRSSLKAYGNGCSSNSNGRTDYTGEGSGCYLGEEKDS..ERLCEDAPGP
B2AR_MOUSE   R.IAFQELLCLRRSSSKTYGNGYSSNSNGRTDYTGEPNTCOLGQEREQ..ELLCEDPFGM
B4AR_MELGA   R.SAFRKLLCCPRRADRRRLHAAPQDPQHCSCAFSPRGDPMEDSKAVDP..GHLREDSE.V
D1A2_EEL     R.KAFSILLCCHRLC....PG.SNAIE.IVSINNNGAPP..QLVHNQ..PKACFSK.GC
D1BR_CARP    R.RAFSSLLRCRTPV....ET.VNASNALVSYNREAASACVNIIPNV..VDETLDRMSQ
D5DR_FUGRU   R.KAFTTILGCSKFCSSSAVQA.VDFSNELVSYHHD.TTLQKEPVPGP..GAHRL..VAP
D1B_AMPHIOXUS R.KVFVKMICCHKCRGVTVGPNHADLNYPVAMRLKKRGENANGTVN..GDANGKANGN
5H4_CAVPO    R.RAFLIILCCDDERYRRPSILGQTVPCSTTTINGSTHVLRTDTECGGQWESQCHPAASS
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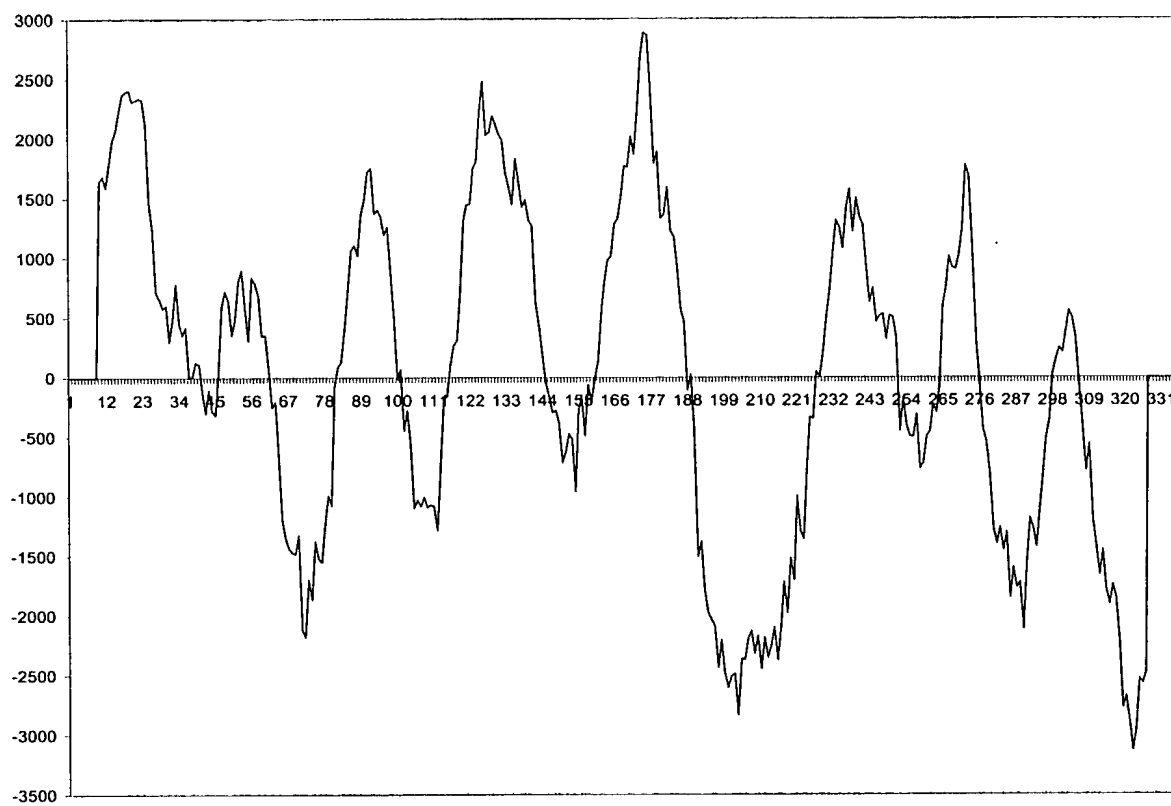
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B2AR_PIG     EGCAHROGTVPDDSTDSQGR...NC..STNDSML~~~~~
B2AR_MOUSE   EGFVNCOGTVPSLSVDSQGR...NC..STNDSPL~~~~~
B4AR_MELGA   QGSGRREENASSHGGGHQQRPLGECWLQGMQSMICEQLDEFTSTEMPAGPSV~~~~~
D1A2_EEL     ..LPKE.CNL.RHGIPHT..ILSQDEELQKKGNAIERISPALSGSLDSEADLSLDKINPT
D1BR_CARP    ..LSRG.GDVLDGAVHANGIL~~~~~
D5DR_FUGRU   ..LPQNRGDA...G.PNFDKVSVVSDDSRADRN...LLPAIL.QCDCEAEISLDMVPFG
D1B_AMPHIOXUS ..TEAGECTSSS~~~~~
5H4_CAVPO    PLVAAQPIDT~~~~~
HGPRBMY26    ~~~~~

B2AR_CANFA  ~~~~~
B2AR_PIG     ~~~~~
B2AR_MOUSE   ~~~~~
B4AR_MELGA   ~~~~~
D1A2_EEL     TQNGQNST~~~~~
D1BR_CARP    ~~~~~
D5DR_FUGRU   SSGPADSFLIPGQIQDLGDL
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5H4_CAVPO    ~~~~~
HGPRBMY26    ~~~~~

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Figure 4

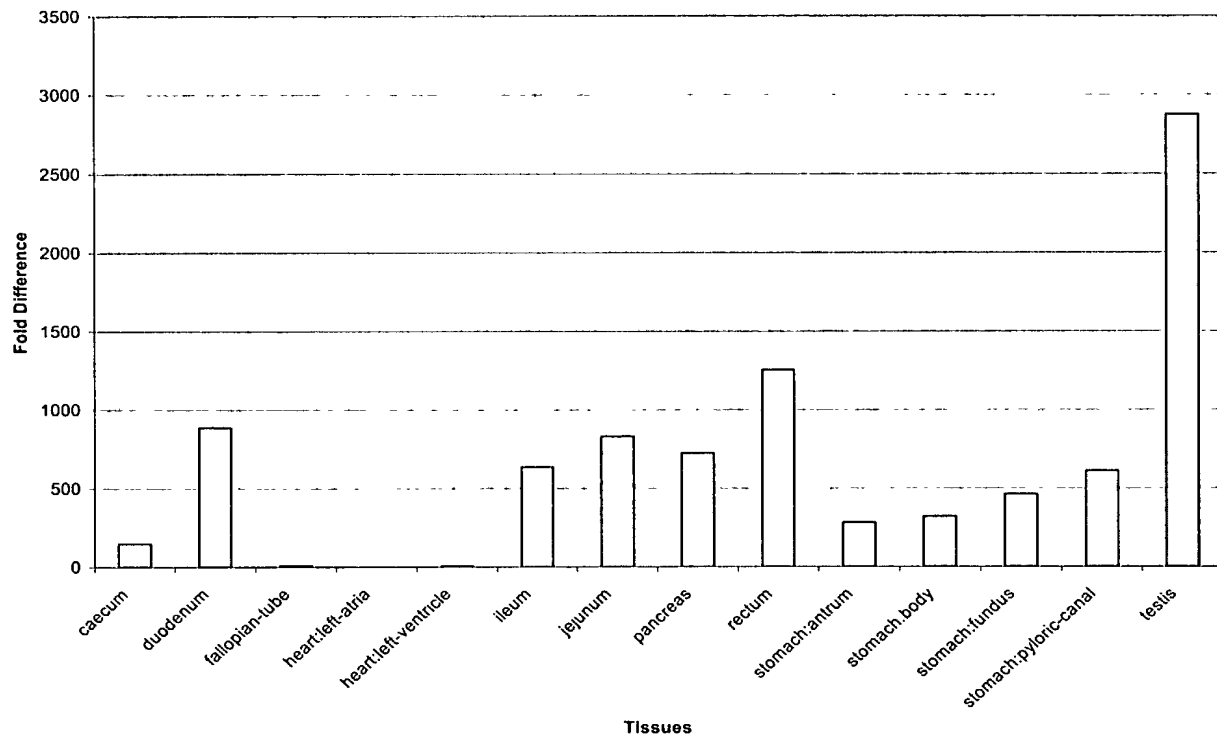


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Figure 5.

<u>Protein</u>	<u>SWISS PROT ID</u>	<u>Identities</u>	<u>Similarities</u>
guinea pig 5-hydroxytryptamine 4 receptor (5-HT-4) protein	gi O70528	32.18%	40.38%
Amphioxus dopamine D1/beta receptor protein	gi O96716	26.28%	35.35%
Fugu rubripes D(5)-like dopamine receptor protein	gi P53454	29.01%	37.35%
carp D1B dopamine receptor protein	gi O42317	27.22%	35.78%
eel dopamine D1A2 receptor protein	gi Q98842	29.31%	39.58%
turkey beta-4C adrenergic receptor protein	gi P43141	29.28%	38.32%
mouse beta-2 adrenergic receptor protein	gi P18762	27.52%	38.23%
pig beta-2 adrenergic receptor protein	gi Q28997	29.57%	39.94%
dog beta-2 adrenergic receptor protein	gi P54833	27.52%	38.53%

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Figure 6

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Figure 7

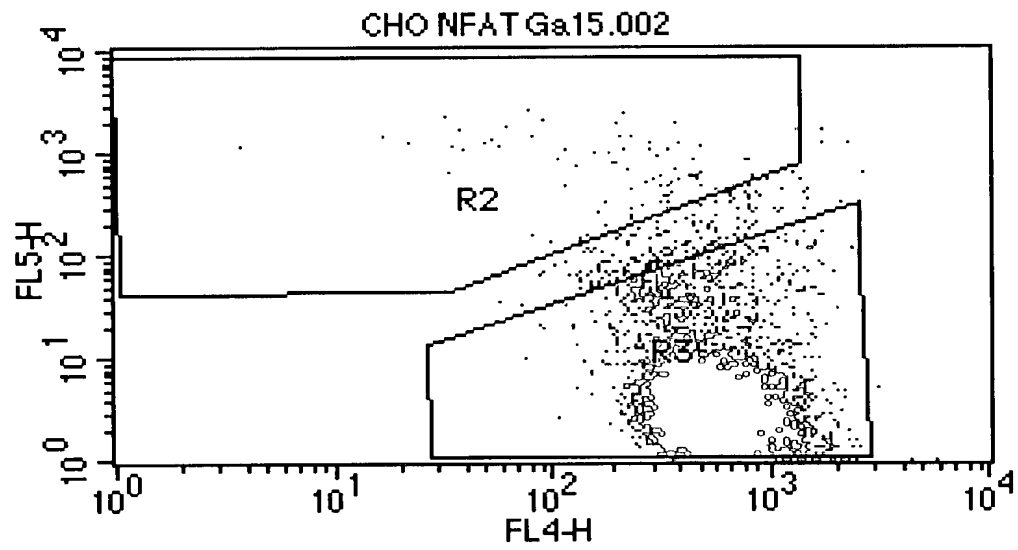
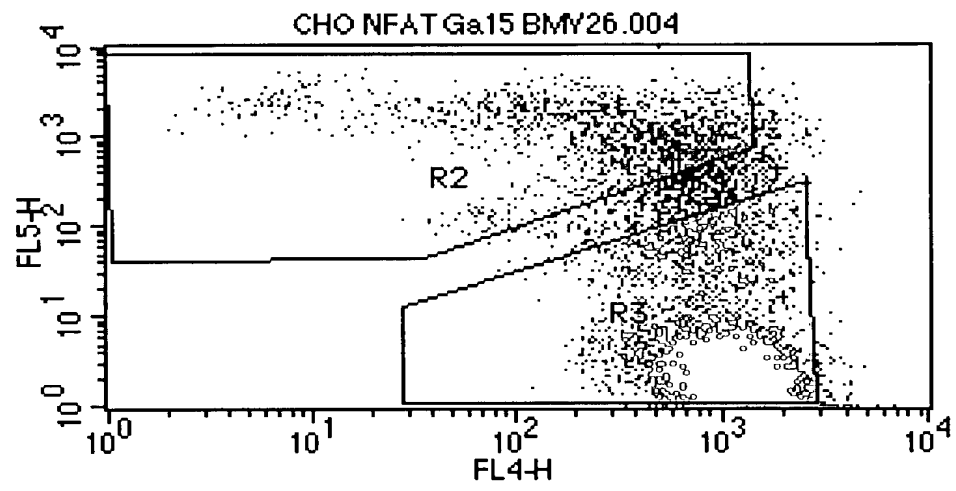
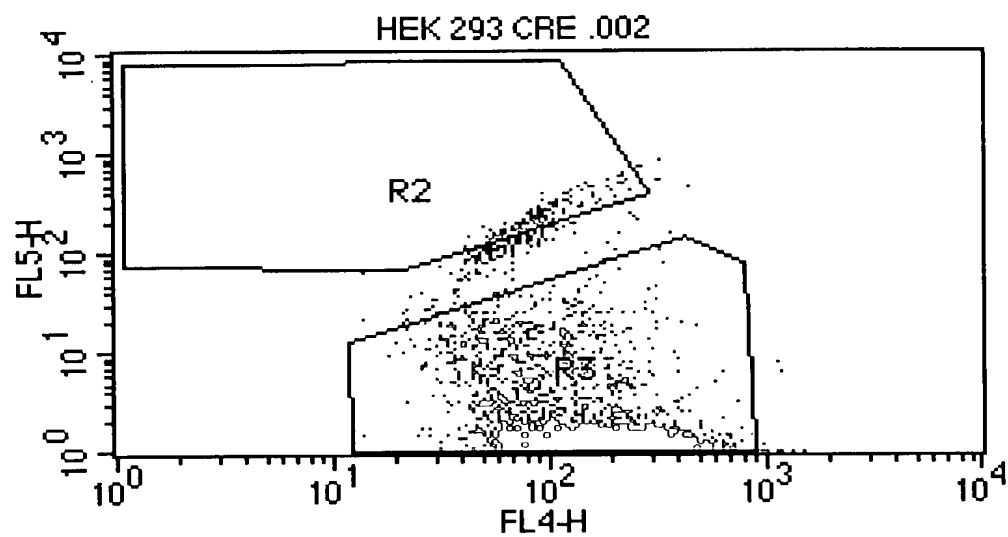


Figure 8



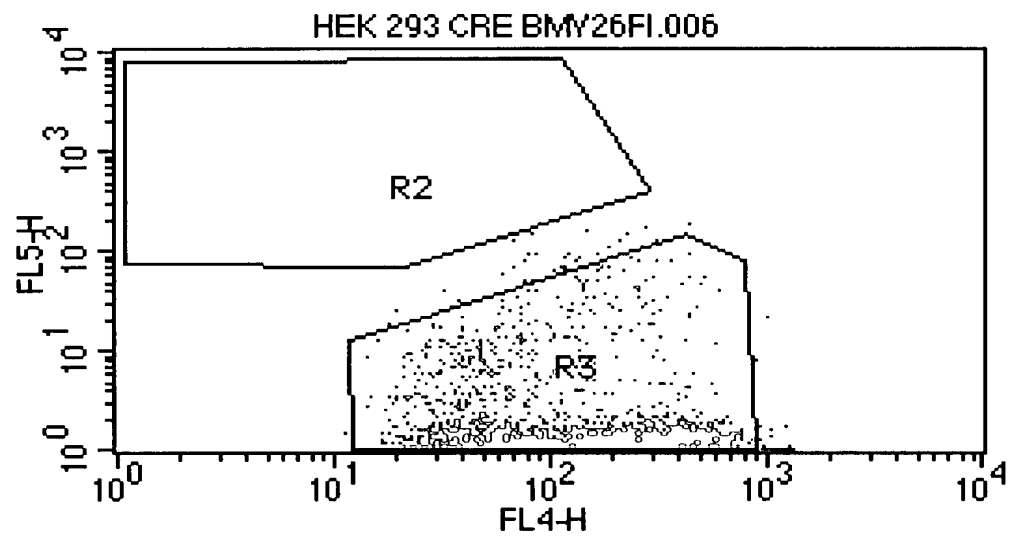
D0128 NP

Figure 9



D0128 NP

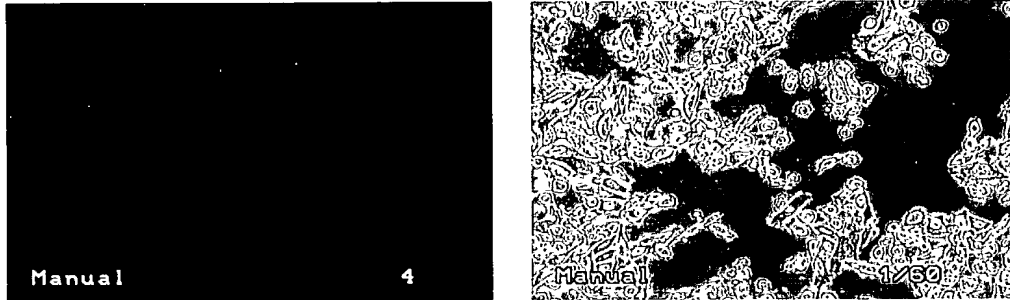
Figure 10



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Figure 11

Cho NFAT Ga15 Control (Fluorescent vs. Bright Field)



Cho NFAT Ga15 BMY26 (Fluorescent vs. Bright Field)

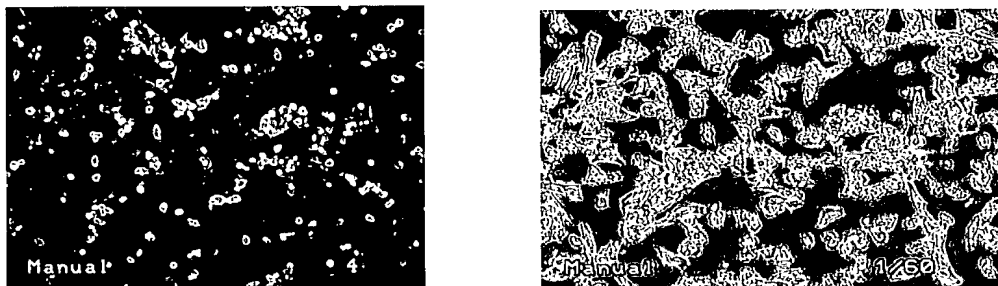
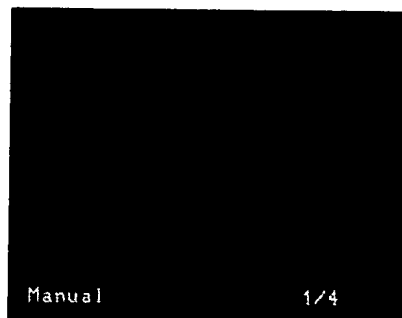
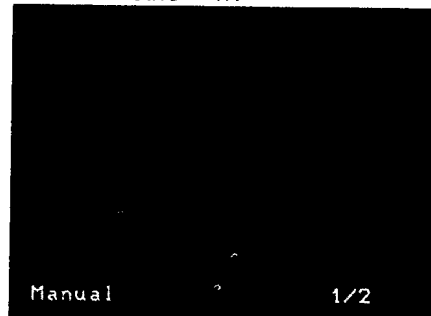


Figure 12

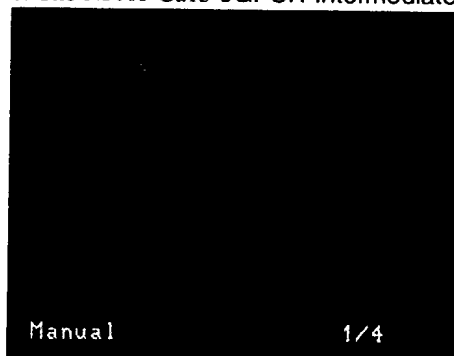
a. Cho NFAT Ga15



b. Cho NFAT Ga15 + T/P



c. Cho NFAT Ga15 oGPCR-Intermediate



d. Cho NFAT Ga15 oGPCR High

